TARC Note

Absence of Detoxification of DHP by Rumen Microorganisms from Malaysian Cattle, Water Buffalos and Sheep

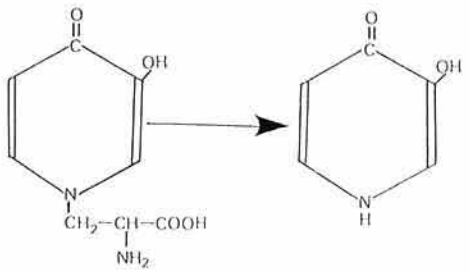
Leucaena is widely grown in tropical and sub-tropical countries. The plant is potentially a source of crude protein and is regarded as a promising forage species. However, its use for feed has been limited because it contains mimosine, β-[N-(3-hydroxy-4-pyridone)]-α-aminopropionic acid, a toxic substance that causes low weight gains, poor health condition and hair loss in both ruminants and non-ruminants[2]. Mimosine is readily hydrolyzed in the rumen of ruminants by microbial degradation to 3-hydroxy-4-(1H)-pyridone, and sometimes to 2-hydroxy-3-(1H)-pyridone (DHP)*, a potent goitrogen[1]. Mimosine toxicity is more acute in nonruminants owing to the absence of endogenous microorganisms capable of enzymatic detoxification. Kudo et al. demonstrated a potential detoxification of mimosine in Canadian domestic ruminants[9] and in voles[10]: rate of mimosine and DHP degradation was enhanced by microbiota from animals fed on concentrate diets, but inocula from animals on hay diets were relatively inactive. Workers in Hawaii, USA[4,5], Indonesia[8] and the Bahamas[12] reported no adverse effects of *Leucaena* fed to beef and dairy cattle, goats and sheep. The absence of toxicity in ruminants in these areas was attributed to the microbial degradation of DHP in the rumen[5,6]. Mimosine toxicity prevents intensive use of *Leucaena* as forage in Australia, Papua New Guinea and probably in Africa, but it has not been reported from much of Asia or tropical America[7]. An *in vitro* test for mimosine and DHP degradation using ruminal contents taken from ruminants in the Selangor area, Malaysia was conducted.

Three Kedah Kelantan (KK) steers and two water buffalos kept at the Faculty of Veterinary Medicine and Animal Sciences, Universiti Pertanian Malaysia (UPM), Selangor, were freely fed Guinea grass. Rumen samples were obtained from rumen fistula for three consecutive days. In addition, four rumen-fistulated KK steers and one male water buffalo kept in the Malaysian Agricultural Research and Development Institute (MARDI) were used for the study. They were fed Guinea grass. Inocula were taken from six groups of sheep (Dorset × local crossbred) fed different diets. Namely group 1 fed Guinea grass (98%), group 2 fed palm kernel cake (83%) + bran (15%), group 3 fed palm kernel cake (97%) + urea (0.5%), group 4 fed palm kernel cake (97%) + urea (1%), group 5 fed palm kernel cake (82%) + Guinea grass (15%) + urea (0.5%), and group 6 fed palm kernel cake (82%) + Guinea grass (15%) + urea (1%). Each group was made of two animals except group 6 (one animal). All animals received 2 to 2.5% mineral supplements. Rumen samples of them were taken through a stomach tube (0.8 cm in internal diameter) 1 hour after the morning feeding.

Mimosine (Sigma Chemical Co.) and 2,3-dihydroxy pyridine (DHP, Aldrich Chemicals Co.) were dissolved in distilled water by heating and stirring. Experimental procedures and estimation of mimosine and DHP were described previously[9]. Mimosine and DHP concentrations were measured at 0, 5 and 24 hr (occasionally, 48 and 72 hr) after incubations.

Rumen contents taken from seven KK cattle, three water buffalos and eleven sheep showed only a little activity of mimosine degradation (mean 5.3%, range 0–9.2%) in 24 hr. Two KK cattle of UPM and one KK cattle of MARDI showed no indication of degradation of mimosine and DHP. In the colorimetric assay, a decrease in absorbance of $> 10\%$ at 535 nm indicated disruption of the 3-hydroxy-4-keto moiety of the pyridine ring and therefore removal of toxic activity in animals[3] but

* Known as 3,4-dihydroxy pyridine (3,4 DHP), and 2,3-dihydroxy pyridine (2,3 DHP).



Mimosine 3-Hydroxy-4(1H)-pyridone (DHP)

a decrease in absorbance of $< 10\%$ could be solely attributed to the hydrolysis of mimosine to DHP and the resultant change in molar extinction coefficient[11]. Therefore, all the 31 samples from 21 animals showed no disruption of the active site on the ring.

The lower rates of disappearance of mimosine measured by the colorimetric method with inocula from the animals fed Guinea grass suggest an absence of an effective combination of microorganisms capable of mimosine and DHP detoxification. This was confirmed with an *in vitro* DHP degradation test using the same samples. None of them showed obvious disruption of the active site on the ring ($< 2\%$). As there is no difference in mimosine degradation rate between 5 and 24 hr samples, conversion of mimosine to DHP seems to occur within 5 hr after incubation. Even after 72 hr there was no indication of any degradation. The result that both mimosine and 2,3-dihydroxy pyridine were not detoxified indicates that 3-4-dihydroxy pyridine was also not detoxified.

From these results it is obvious that the tested Malaysian ruminants may be able to convert mimosine to DHP. But they were unable to degrade DHP, although roughage diets might have account for the lower activity to some extent. A concentrate diet may increase the detoxification and serve to alleviate mimosine poisoning. Selection and breeding of *Leucaena* for low mimosine content may be a solution. However, it seems that the more practical method to overcome the toxicity problem is to transfer specific rumen microorganisms which have high detoxification ability from other countries where such microorganisms exist. Development of the viable bacterial preparation that degrades and detoxifies mimosine and DHP is desired.

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